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Research Report

Potential output pathways for agonistic-like responses resulting from the $GABA_A$ blockade of the torus semicircularis dorsalis in weakly electric fish, *Gymnotus carapo*

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ABSTRACT

The purpose of this study is to examine the pathways involved in the electromotor (electric organ discharge interruptions) and skeletomotor responses (defense-like) observed by blockade of GABAergic control of the torus semicircularis dorsalis (TSd) of the awake weakly electric fish *Gymnotus carapo*, described in a former study. Microinjection of NMDA (5 mM) into the pacemaker nucleus (PM) through a guide cannula previously implanted caused a prolonged interruption of the electric organ discharge (EOD) intermingled with reduction in frequency, similar to that described for TSd $GABA_A$ blockade, but without noticeable skeletomotor effects. The EOD alterations elicited by bicuculline microinjections (0.245 mM) into the TSd could be blocked or attenuated by a previous microinjection of AP-5 (0.5 mM), an NMDA antagonist, into the PM. Labeled terminals are found in the nucleus electrosensorius (nE) after injection of the biotinylated dextran amine (BDA) tracer into the TSd and into the sublemniscal prepacemaker nucleus (SPPn) subsequent to the tracer injection into the nE. Defense-like responses but not EOD interruptions are observed after microinjections of NMDA (5 mM) into the rhombencephalic reticular formation (RF), where labeled terminals are seen after BDA injection into the TSd and somata are filled after injection of the tracer into the spinal cord. In this last structure, marked fibers are seen subsequent to injection of BDA into the RF. These results suggest that two distinct pathways originate from the torus: one for EOD control, reaching PM through nE and SPPn, and the other one for skeletomotor control reaching premotor reticular neurons. Both paths could be activated by toral $GABA_A$ blockade.

1. Introduction

Evidence was provided, in a former work (*Brazilian Journal of Medical and Biological Research*, accepted for publication) for the involvement of the torus semicircularis dorsalis (TSd) of *Gymnotus carapo* in defense since the inhibition of local GABAergic control resulted in a strong skeletomotor response consisting of recognizable agonistic patterns and in EOD interruptions, known to occur in agonistic encounters.

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In the gymnotiforms *Eigenmannia* and *Apteronotus*, toral efferents arise mainly from layers VI to IX and reach among others the optic tectum (TeO), the diencephalic nucleus electrosensorius (nE), the isthmic nucleus praeminentialis and the reticular formation (RF) (Carr et al., 1981). The torus has therefore direct access to spinal motor neurons through RF and indirect access to electromotor precommand neurons through nE (Bastian and Yuthas, 1984; Carr et al., 1981; Keller, 1988; Metzner, 1993). This last structure interfaces the electrosensory processing executed in the TSd with the premotor control of the pacemaker nucleus (PM) via the thalamic central posterior/prepacemaker complex (CP/PPn) (Heiligenberg et al., 1981; Kawasaki and Heiligenberg, 1988; Kawasaki et al., 1988; Metzner, 1993; Rose et al., 1988; Zupanc and Heiligenberg, 1992) or via the mesencephalic sublemniscal nucleus (SPPn) (Heiligenberg et al., 1996; Kawasaki and Heiligenberg, 1988; Keller et al., 1991; Metzner, 1993). The tectum provides another toral efferent pathway for controlling electro and skeletomotor behaviors since it projects to the CP/PPn complex (Zupanc and Horschke, 1996) and also to the reticular formation (RF) (Heiligenberg and Rose, 1987).

In teleosts, the cells of origin of descending pathways to the spinal cord are exclusively located in the rhombencephalic RF, octavolateral region and in the mesencephalic tegmentum. These projections descend through the so-called tractus bulbospinalis (bs), located dorsolaterally in the funiculus lateralis (fl), the tractus vestibulospinalis (vesp), located ventrolaterally in the fl, and the medial longitudinal fasciculus (MLF) that runs in the funiculus ventralis (fv). The MLF is predominantly composed of reticulospinal fibers, including those of the Mauthner cells (Meek and Nieuwenhuys, 1998). The reticular formation is a rather undifferentiated region of the ventral and intermedioventral rhombencephalic zone and is subdivided into a median zone containing the raphe neurons, a large medial zone located ventrally and a small lateral zone located intermedioventrally. Whereas the median and medial zones continue rostralward in the mesencephalon, the lateral zone is restricted to the rhombencephalon (Nieuwenhuys and Pouwels, 1983). The medial zone possesses large and medium-sized neurons that project to the spinal cord in *Anguilla* (Bosch and Roberts, 1994), *Carassius* (Rao Prasada et al., 1987), *Eigenmannia* (Behrend and Donicht, 1990), and *Brachydanio* (Lee and Eaton, 1991) and that project to the pituitary in *Apteronotus* (Corrêa and Zupanc, 2004). Thus, reticular formation cells are directly linked to motor and endocrine functions.

This study aims to propose the pathways mediating the skeletomotor and electromotor effects resulting from the blockade of the toral GABAergic control described in a previous study and recognized as components of the agonistic repertoire of *G. carapo*. For this purpose, a pharmacological approach was used, involving the microinjections of drugs related to the GABAergic and glutamatergic systems to elicit these responses and of the respective agonists or antagonists to examine the specificity of the effects. A neuroanatomical "in vivo" tract tracing technique was employed to reconstruct the underlying neural pathways. Conscious unrestrained animals with implanted cannulae for intracerebral injections were privileged for the functional approach. The working hypothesis considered the reticular formation involvement in the somatic effects and of the nE and its projections to PM via the SPPn in EOD interruptions. As a first step, evidence is provided for the glutamatergic mediation of the EOD interruptions at the PM in *G. carapo*, similarly to what is described using other approaches (iontophoretic injections in immobilized animals) in other gymnotiformes (Dye et al., 1989; Kawasaki and Heiligenberg, 1989; Zupanc and Heiligenberg, 1992).

2. Results

2.1. *Physiological data*

The effects of microinjections of NMDA (5 mM), or AP-5 (0.5 mM) into the ventral PM on the EOD frequency, are seen in Fig. 1A. Microinjections of AP-5 did not interfere with the EOD, and the distribution of its mean values is similar to the mean values of saline. In contrast, microinjections of NMDA resulted in sudden and long-lasting EOD interruption intermingled with resumes of activity or decreased frequencies, which was already established at time 1.5 min after the microinjection of the drug and lasted up to the end of the experiment. At 1.5, 5, 10, 15, 20, 25, and 30 min, the EOD frequencies values (0 Hz; 6.4 ± 4.3 Hz; 6.1 ± 4.1 Hz; 14 ± 4.9 Hz; 19.6 ± 3.4 Hz; 22 ± 2.8 Hz and 21.3 ± 2.8 Hz, respectively) were statistically different from the control value (29.2 ± 1.4 Hz) ($P < 0.05$, ANOVA followed by Tukey's test) and from the values of saline at the same times (30.3 ± 1.6 Hz; 31.1 ± 1.6 Hz; 31.1 ± 1.6 Hz; 30.4 ± 1.5 Hz; 31.7 ± 1.9 Hz; 30.5 ± 1.6 Hz; 31.1 ± 1.9 Hz, respectively) ($P < 0.05$, ANOVA followed by Dunnett's method).

The effects of the microinjection of the $GABA_A$ antagonist bicuculline (0.245 mM) into the deep layers of the torus semicircularis dorsalis (TSd) on the EOD frequency, are seen in Fig. 1B. Microinjections of bicuculline resulted in the occurrence of sudden and temporary EOD interruptions or decreased frequencies that was maximum at 1.5 min after the microinjection and lasted up to 15 min. At the times 1.5, 5, 10, and 15 min, the EOD frequencies values (0 Hz, 6 ± 4.2 Hz; 13 ± 6.0 Hz; 20.7 ± 5.5 Hz, respectively) were statistically different from the control value (34.4 ± 2.9 Hz) ($P < 0.05$, ANOVA followed by Tukey's test). Microinjections of bicuculline into the TSd preceded by microinjections of AP-5 into the PM did not produce EOD interruptions but decreased EOD frequencies that, at the times 1.5, 5, 15, 20, 25, and 30 min had values (23.3 ± 4.0 Hz; 23.3 ± 4.0 Hz; 24.7 ± 3.5 Hz; 24.1 ± 3.4 Hz; 23.7 ± 3.2 Hz; 24.5 ± 3.7 Hz, respectively), statistically different from the control value (32.7 ± 3.3 Hz) ($P < 0.05$, ANOVA followed by Tukey's test). At the times 1.5 and 5 min, the EOD frequencies values (23.3 ± 4.0 Hz; 23.3 ± 4.0 Hz) were statistically different from the values of 0 Hz and 6 ± 4.2 Hz, after the microinjection of only bicuculline into the TSd at the same times ($P < 0.05$, ANOVA followed by Tukey's test) (Fig.B).

Fig. 2 shows the effect of microinjections of NMDA (5 mM) or saline into the rhombencephalic RF on the EOD frequency during the experimental time. Microinjections of NMDA resulted in increased EOD frequencies and at 10 min after the microinjection the EOD frequency value (42.5 ± 4.4 Hz) was statistically different from its control value (31.8 ± 3.5 Hz)